Claims

1. A wound healing composition comprising isolated living cells having a wound healing phenotype, characterised in that the cells of the composition:

(i) exhibit a 2 to 48000-fold, more preferably a 100 to 2000-fold, higher level of expression of apolipoprotein D (ApoD) than of Ribosomal protein L32 (RPL32);
exhibit a 2000 to 1600000-fold, more preferably a 13000 to 100000-fold, higher level of expression of matrix metalloprotease 2 (MMP2) than of RPL32;
exhibit a 20 to 44000-fold, more preferably a 800 to 1800-fold, higher level of expression of collagen 3a1 (Coll3a11) than of RPL32; and
exhibit a 20 to 150000-fold, more preferably a 1600 to 2500-fold, higher level of expression of smooth muscle actin (SMA) than of RPL32; and/or

(ii) have a banding pattern of polymerase chain reaction (PCR) products resulting from differential display identical or similar to that shown in Fig. 4 or Fig. 5 for nucleic acid expression in fibrin.

2. The wound healing composition according to claim 1, in which the cells further exhibit a 1 to 500-fold, more preferably a 13 to 160-fold, higher level of expression of "X-ray repair, complementing defective, in Chinese hamster, 1" (DD5) than of RPL32; and/or exhibit a 1 to 210-fold, more preferably a 3 to 15-fold, higher level of expression of a gene deposited as Genbank Accession No. gi|10437022 (DD10) than of RPL32; and/or exhibit a 1 to 33-fold, preferably a 1 to 5-fold, higher level of expression of a gene deposited as Genbank Accession No. gi|12410897 (GB1) than of RPL32.

3. The wound healing composition according to any preceding claim, in which the composition after development of the wound healing phenotype is maintained at a temperature of between about 20°C to 42°C, preferably about 37°C, and in which the cells further exhibit a 1000 to 120000-fold, preferably a

11000 to 53000-fold, higher level of expression of ribosomal protein S24 (GB5), and/or exhibit a 120 to 36000-fold, preferably a 1000 to 30000-fold, higher level of expression of ribosomal protein S8 (DD12) than of RPL32, and/or exhibit a 0 to 750000-fold, more preferably a 1 to 136000-fold, higher level of expression of a gene deposited as Genbank Accession No. gi|7022020 (DD2) than of RPL32.

4. The wound healing composition according to any preceding claim, in which the composition after development of the wound healing phenotype is stored at a temperature of 2°C to 8°C, for example 3°C to 5°C, preferably about 4°C, and in which the cells further exhibit a 130 to 760-fold higher level of expression of urokinase (PLAU), and/or exhibit a 28000 to 2065000-fold higher level of expression of vimentin (Vim) than of RPL32.

5. The wound healing composition according to any preceding claim, in which the living cells are incubated within a protein-rich environment for up to about 14 days to allow development of the wound healing phenotype.

6. The wound healing composition according to claim 5, in which the protein-rich environment comprises any of the group consisting of fibrin, collagen, fibronectin, vitronectin, alginate, agar, hyaluronic acid, modified starches, carrageenans, carob, gelatine, pectin and gelling agents.

7. The wound healing composition according to either of claim 5 or claim 6, in which the protein-rich environment is a support matrix.

8. The wound healing composition according to claim 7, in which the cells are suspended within the matrix, preferably substantially uniformly within the matrix.

9. The wound healing composition according to either of claim 7 or claim 8, in which the matrix is protein-based, for example having a protein

concentration in the range of about 3 to 12 $mg.ml^{-1}$.

10. The wound healing composition according to any of claims 7 to 9, in which the matrix is a fibrin matrix.

11. The wound healing composition according to claim 10, in which the fibrin has a concentration in the range of 3 to 12 $mg.ml^{-1}$, for example 7 to 12 $mg.ml^{-1}$ or 3 to 5 $mg.ml^{-1}$.

12. The wound healing composition according to either of claim 10 or claim 11, in which the fibrin matrix is formed by thrombin-mediated polymerisation of fibrinogen.

13. The wound healing composition according to any of claims 7 to 12, in which the matrix is non-pyrogenic and/or sterile.

14. The wound healing composition according to any of claims 7 to 13, in which the cells are cast into the support matrix before incubation.

15. The wound healing composition according to any of claims 7 to 14, in which the matrix is solid or semi-solid.

16. The wound healing composition according to any preceding claim, in which the composition is stored for up to about 40 days, preferably up to 19 days and more preferably about 7 to 14 days or about 7 to 11 days at a temperature of 2°C to 8°C, for example 3°C to 5°C, preferably about 4°C, while retaining the wound healing phenotype.

17. The wound healing composition according to any preceding claim, in which the cells are mammalian, for example human.

18. The wound healing composition according to any preceding claim, in

which the cells are substantially fibroblasts, for example 90% to 100%, preferably 95% to 99.5%, and more preferably 97.5% to 99% fibroblasts.

19. The wound healing composition according to claim 18, in which the fibroblasts are dermal fibroblasts, preferably human dermal fibroblasts.

20. The wound healing composition according to any preceding claim, in which the cells substantially exclude keratinocytes.

21. The wound healing composition according to any preceding claim, in which the cells are human dermal fibroblasts within a sterile, non-pyrogenic support matrix formed by thrombin-mediated polymerisation of fibrinogen, and in which the composition has been incubated for 16 to 24 h at about 37°C.

22. A wound healing composition comprising fibroblasts cultured within a fibrin matrix, in which the fibroblasts of the composition have a wound healing phenotype and have a higher level of expression of collagen 6a1 (Coll6a), apolipoprotein D (APOD), collagen 3a1 (Coll3a1), ribosomal protein L32 (RPL32), plasminogen activator inhibitor (PAI), urinary plasminogen activator (PLAU), vimentin (Vim), smooth muscle actin (SMA) and cyclo-oxygenase 2 (Cox2) than fibroblasts cultured in a collagen matrix and fibroblasts cultured in medium without a matrix.

23. The wound healing composition according to claim 22, in which the fibroblasts of the composition have approximately a 3-fold higher level of expression of Coll6a, and/or a 8-fold higher level of expression of APOD, and/or a 80-fold higher level of expression of Coll3a1, and/or a 3-fold higher level of expression of RPL32, and/or a 3-fold higher level of expression than PAI, and/or a 20-fold higher level of expression of PLAU, and/or a 20-fold higher level of expression of Vim, and/or a 5-fold higher level of expression of SMA, and/or a 8000-fold higher level of expression of Cox2, than fibroblasts cultured in a collagen matrix.

24. The wound healing composition according to either of claim 23 or 24, in which the fibroblasts of the composition have approximately a 4-fold higher level of expression of Coll6a, and/or a 4-fold higher level of expression of APOD, and/or a 10-fold higher level of expression of Coll3a1, and/or a 2-fold higher level of expression of RPL32, and/or a 3-fold higher level of expression than PAI, and/or a 30-fold higher level of expression of PLAU, and/or a 10-fold higher level of expression of Vim, and/or a 2-fold higher level of expression of SMA, and/or a 5000-fold higher level of expression of Cox2, than fibroblasts cultured in medium without a matrix

25. The wound healing composition according to any of claims 22 to 24, in which the fibroblasts of the composition have a higher level of expression of matrix metalloprotease 2 (MMP2), insulin induced gene 1 (INSIG1), growth arrest specific gene 6 (Gas6) and collagen 1a1 (Coll1a) than fibroblasts cultured in a collagen matrix.

26. The wound healing composition according to claim 25, in which the fibroblasts of the composition have approximately a 2-fold higher level of expression of MMP2 and/or INSIG1 and/or Gas6 and/or Coll1a than fibroblasts cultured in a collagen matrix.

27. The wound healing composition according to any one of claims 22 to 26, in which the fibroblasts of the composition have a higher level of expression of glyeraldehyde-3-phosphate dehydrogenase (GAPDH) than fibroblasts cultured in medium without a matrix.

28. The wound healing composition according to claim 27, in which the fibroblasts of the composition have approximately a 3-fold higher level of expression of GAPDH than fibroblasts cultured in medium without a matrix.

29. The wound healing composition according to any preceding claim, in

which the composition is incubated for up to about 8 days, preferably about 96 h, for example up to 72 h, 48 h, 25 h or 24 h, and more preferably for 16 h to 24 h, to allow development of the wound healing phenotype.

30. The wound healing composition according to any preceding claim, in which the composition is incubated at a temperature of about 37°C to allow development of the wound healing phenotype.

31. The wound healing composition according to any preceding claim, in which the cells are actively synthetic or able to become actively synthetic rapidly.

32. The wound healing composition according to any preceding claim, in which the cells are not proliferating and/or not senescent.

33. The wound healing composition according to any preceding claim, further comprising a protease inhibitor, for example aprotinin and/or tranexamic acid.

34. The wound healing composition according to one preceding claim, in which the composition has a thickness of approximately 8 mm or less, preferably 5 mm or less.

35. The wound healing composition according to any preceding claim, comprising about 450 to 2500 cells per mm^2, for example about 750 to 2000 cells per mm^2, preferably about 900 to 1700 cells per mm^2 such as about 1500 cells per mm^2, or for example about 450 to 550 cells per mm^2 and preferably about 500 cells per mm^2.

36. The wound healing composition according to any preceding claim, in which the composition is single-layered.

37. The wound healing composition according to any preceding claim, in which the composition is packaged in a container suitable for transporting the composition (for example, while storing the composition) and/or topically applying the composition to a skin surface.

38. The wound healing composition according to claim 37, in which the container comprises a flexible pouch consisting of two sheets of impermeable flexible material peripherally sealed to provide a means of containment for the composition, the pouch comprising a first internal surface to which the composition is adherent at a level of adhesion more than between the composition and a second internal surface of the pouch but less than that between the composition and the skin surface, such that in use the pouch may be opened by parting the sheets and the composition conveniently manipulated and directly applied to the skin surface without further requirement for the composition to be touched directly by any other means prior to application.

39. The wound healing composition according to either of claim 37 or claim 38, in which the container is an Oliver (RTM) Products Company "Solvent Resistant Peelable Pouching Material" (Product number Q15/48BF1).

40. The wound healing composition according to any preceding claim, for use as a medicament.

41. The wound healing composition according to any preceding claim, for use as a medicament in the treatment of a skin lesion.

42. The wound healing composition according to either of claim 40 or 41, for topical application to a skin lesion such as a venous ulcer, diabetic ulcer, pressure sore, burn or iatrogenic grating wound.

43. A method of manufacturing a wound healing composition as defined in any of claims 5 to 42, comprising the steps of:

suspending living cells in a protein-rich environment; and
incubating the cells under conditions which allow development of a wound healing phenotype in the cells, thereby forming the wound healing composition.

44. The method according to claim 43, in which the cells are suspended in a solution comprising a polymerisation agent and/or a monomer capable of being polymerised by the polymerisation agent into a matrix, and in which the method comprises a further step of forming a single-layered support matrix comprising the cells by polymerisation of the monomer with the polymerisation agent prior to incubating the cells.

45. The method according to claim 44, in which the matrix is formed by adding monomer or polymerisation agent to the solution such that both monomer and polymerisation agent are present in sufficient concentrations to effect polymerisation.

46. A method of manufacturing a wound healing composition as defined in any of claims 7 to 42, comprising the steps of forming a single-layered support matrix by polymerising a polymerisable monomer with a polymerisation agent, casting living cells into the support matrix, and incubating the matrix under conditions which allow development of a wound healing phenotype in the cells, thereby forming the wound healing phenotype.

47. The method according to any of claims 44 to 46, in which the monomer is fibrinogen and the polymerisation agent is thrombin.

48. The method according to any of claim 44 to 47, in which polymerisation occurs in a mould.

49. The method according to any of claims 43 to 48, comprising the further step of packaging the wound healing composition into a container for storing the composition and/or for transporting the composition and/or for topically

applying the composition to a skin surface of a patient.

50. Use of living cells as defined in any of claims 1 to 42 in the manufacture of a wound healing composition as defined in any of claims 1 to 42 for the treatment of a skin lesion.

51. A method of treating a patient suffering from a skin lesion comprising topically applying of a wound healing composition as defined in any of claims 1 to 42 to the skin lesion.

52. A method of determining whether a composition comprising living cells has a wound healing phenotype, comprising the steps of:
(i) quantifying the cellular expression of genes as defined in any of claims 1 to 4; and
(ii) comparing expression level of the genes compared to expression level of RPL32, thereby determining whether the composition has a wound healing phenotype.

53. A method of determining whether a composition comprising living fibroblast cells within a fibrin matrix has a wound healing phenotype, comprising the steps of:
(i) quantifying the expression of genes as defined in any of claims 22 to 28 in the cells of the composition and in fibroblasts cultured in a collagen matrix and in fibroblasts cultured in medium without a matrix; and
(ii) comparing expression level of the genes, thereby determining whether determining whether the composition has a wound healing phenotype.

54. A method for conducting a business, comprising the step of determining whether a composition has a wound healing phenotype according to the method of claim 52 and/or claim 53.